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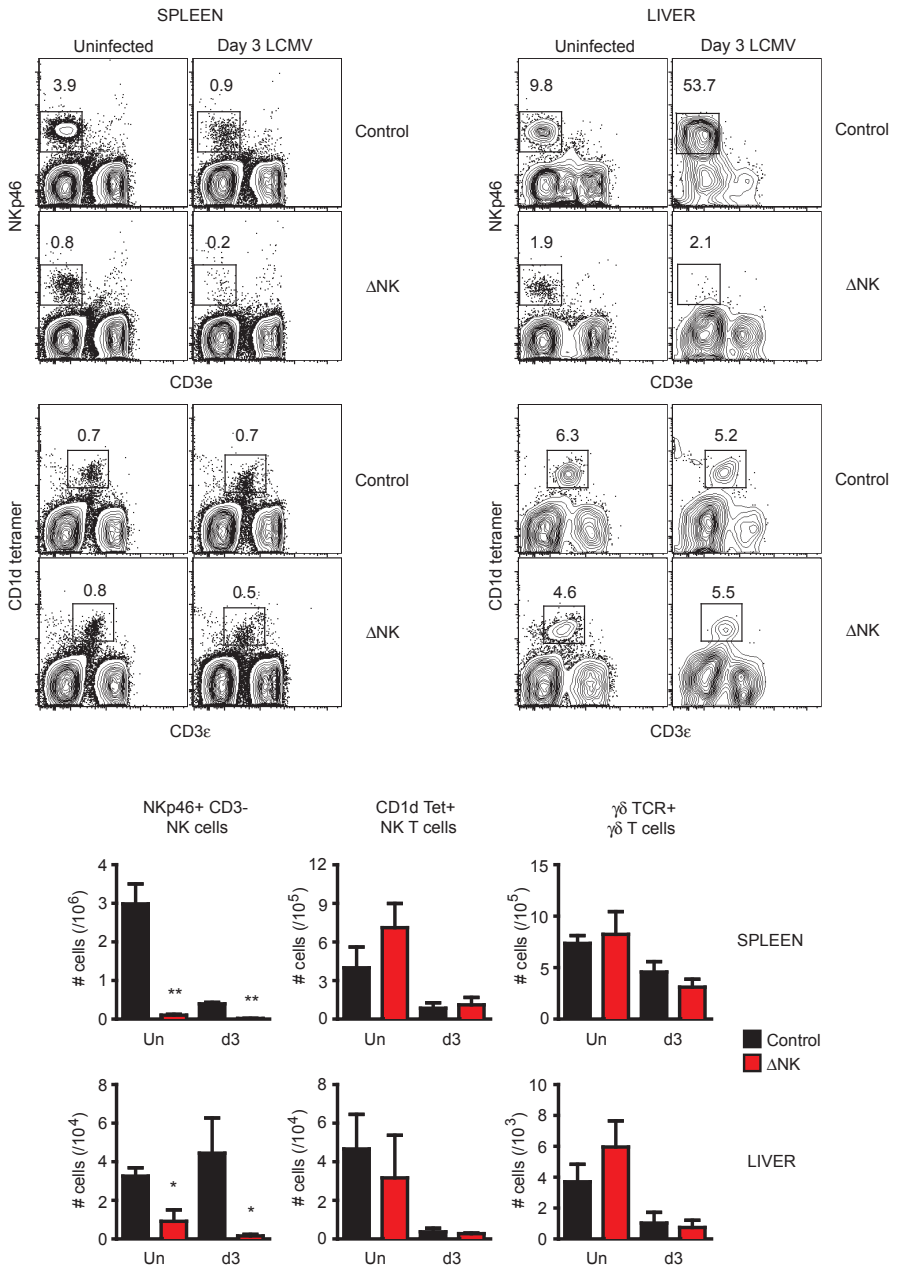
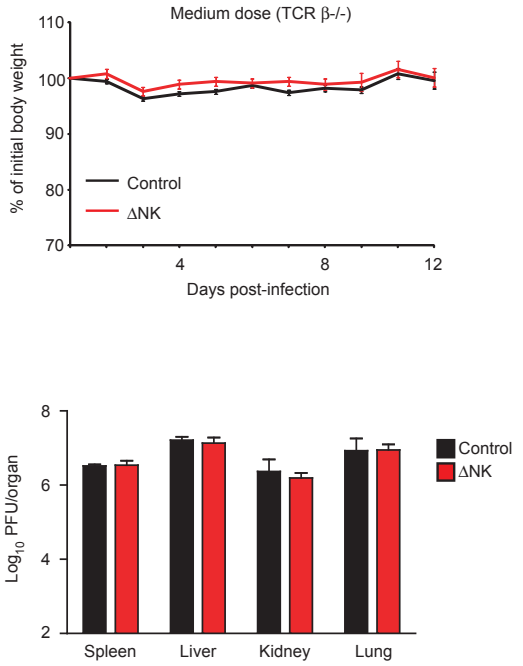
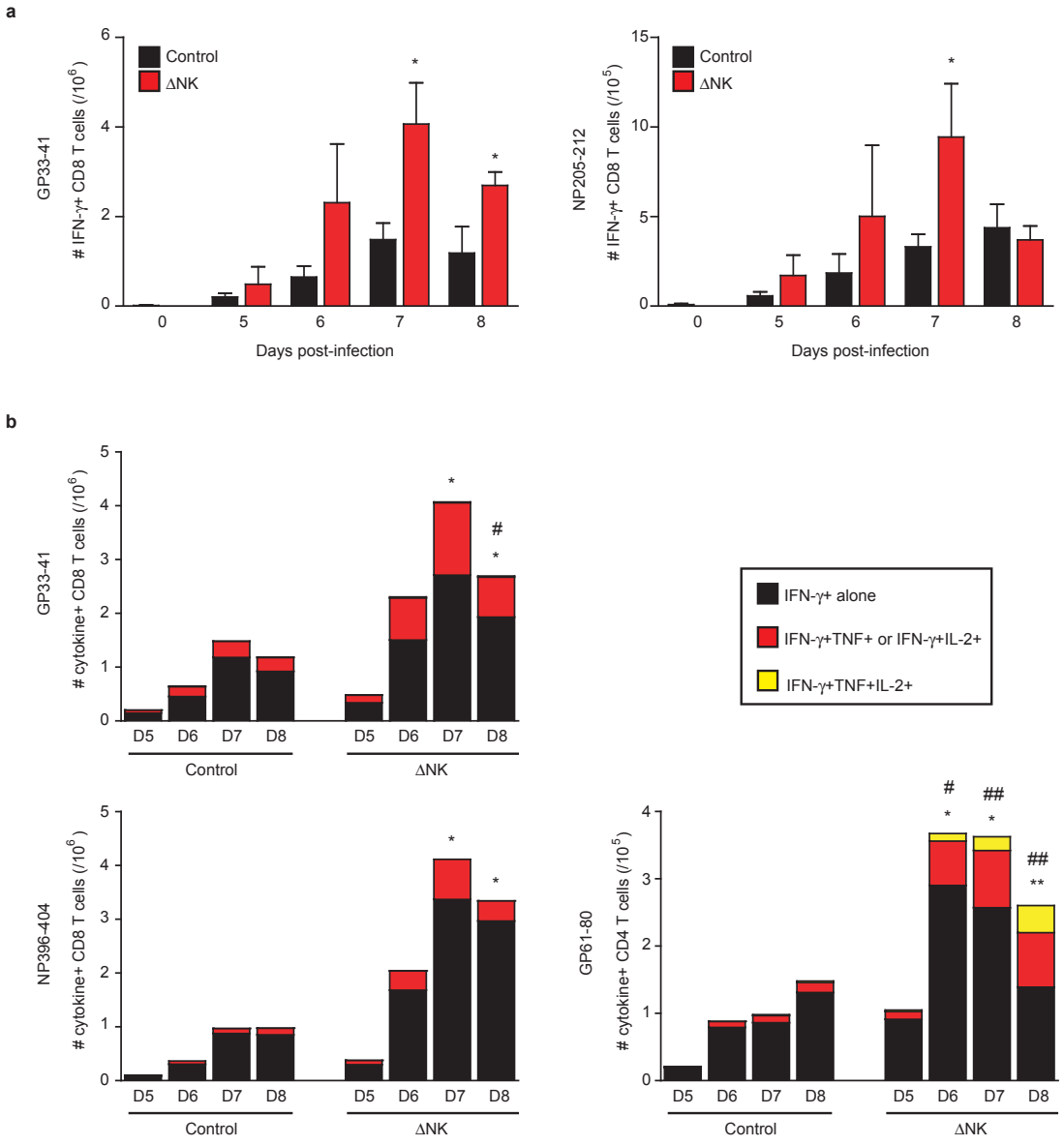


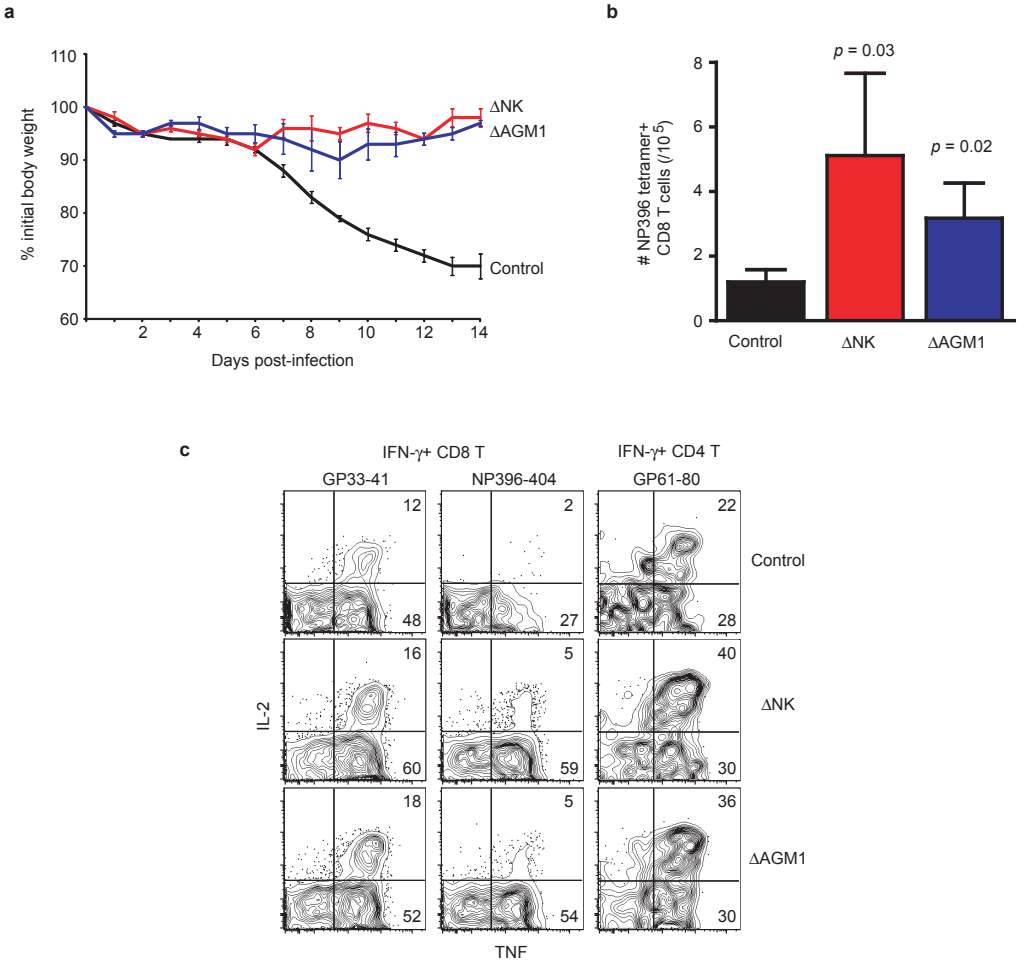
Supplemental Figure 1. Low dose of anti-NK1.1 mediates specific depletion of NK cells in LCMV-infected mice. Groups of mice (n=3/group) treated with 25 μg IgG2a isotype (Control) or anti-NK1.1 (ΔNK) were analyzed four days later (uninfected) or were given a medium dose (2 x 10^5 PFU) of LCMV and analyzed on day 3 of infection (day 3 LCMV). Splenocytes and liver lymphocytes were stained for cell surface makers in order to distinguish NK cells ($CD3^{neg}$ $NKp46^+$), NK T cells ($CD3^+$ CD1d $tetramer^+$), and γδ T cells ($CD3^+$ γδ TCR^+). Total numbers of individual cell types were calculated in each organ and plotted as mean±s.e.m. Significant differences between control and NK cell-depleted mice are denoted: *p<0.05, **p<0.01.



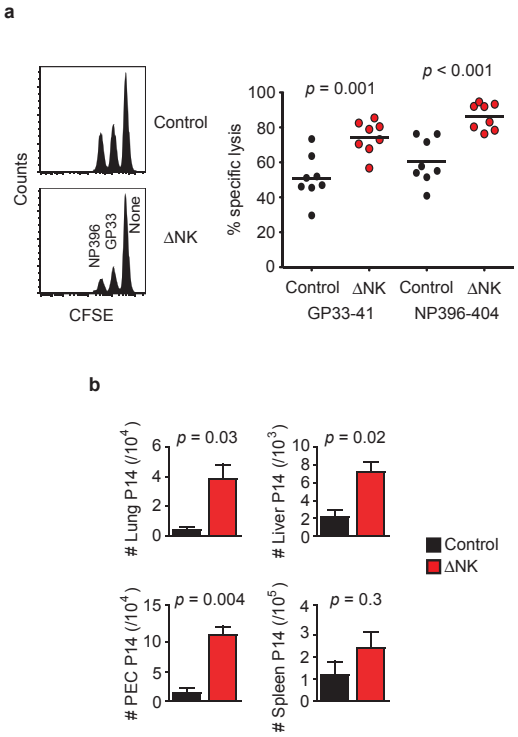
Supplemental Figure 2. Role of T cells in medium dose pathology and enhanced viral control in absence of NK cells. Weight loss and viral load at day 15 p.i. in αβ T cell-deficient (TCR β-/-) mice (n=6/group) treated with IgG2a (control) or anti-NK1.1 (ΔNK) i.p. one day prior to i.v. infection with a medium dose (2 x 10^5 PFU) of LCMV clone 13. Results presented as mean±s.e.m.



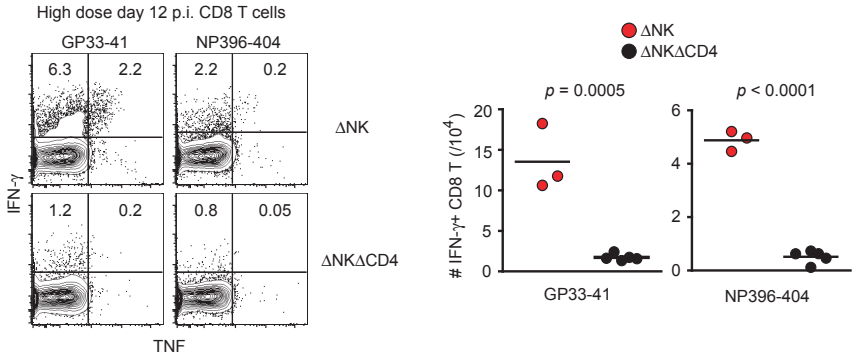
Supplemental Figure 3. Frequency and number of LCMV-specific CD8 T cells are enhanced in NK cell-depleted mice during medium dose infection. Splenocytes were harvested from isotype-treated (Control) or anti-NK1.1 treated (ΔNK) mice at various days after infection with medium dose (2 x 10^5 PFU i.v.) LCMV clone 13 infection and (**a**) IFN-γ production by CD8 T cells was analyzed by intracellular cytokine staining after a 5 hour in vitro stimulation with viral peptide or anti-CD3 antibody. Total numbers of LCMV-specific IFN-γ$^+$ CD8 T cells in the spleen are plotted as mean±s.e.m. (n=3/group/time). **b**, IFN-γ$^+$ CD8 and CD4 T cells were further analyzed for co-production of TNF and IL-2. The total number of LCMV-specific T cells producing one (IFN-γ), two (IFN-γ and TNF or IL-2), or three cytokines (IFN-γ and TNF and IL-2) is plotted as mean±s.e.m. (n=3/group/time). Significant differences between control and NK cell-depleted mice with regards to the number of (**a**) IFN-γ$^+$ or (**b**) IFN-γ/TNF double-positive T cells are denoted: *p<0.05, **p<0.01. Significant differences in the number of (**b**) IFN-γ/TNF/IL-2 triple-positive T cells are denoted: $^{\#}p$<0.05, $^{\#\#}p$<0.01.



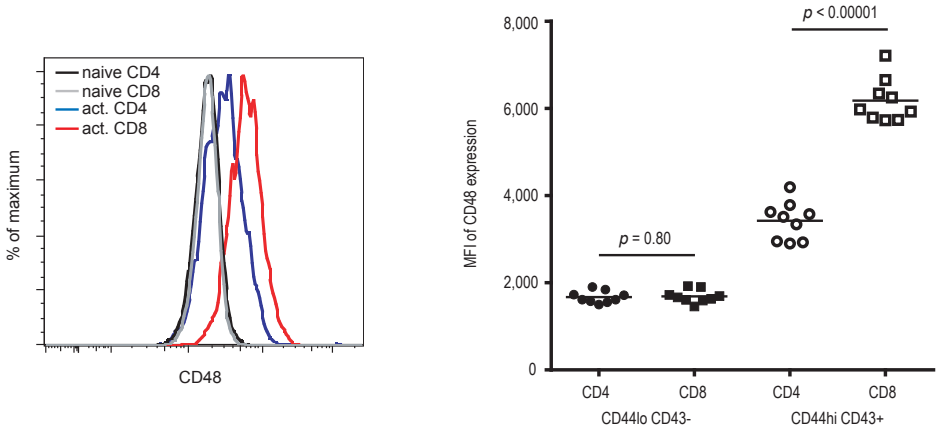
Supplemental Figure 4. Depletion of NK cells with anti-asialoGM1 enhanced anti-viral T cell responses and viral clearance. Groups of mice (n=3/group) were treated with isotype (Control), 25 μg anti-NK1.1 (ΔNK), or a titrated dose (10 μL) of anti-asialoGM1 (ΔAGM1) i.p. one day prior to infection with a medium dose (2 x 10^5 PFU) of LCMV i.v. **a**, Weight loss (mean±s.e.m.) was monitored daily. **b,** Number (mean±s.e.m.) of LCMV-specific CD8 T cells was determined in spleen at day 6 p.i. by LCMV peptide-loaded MHC class I tetramer staining. **c**, Co-production of TNF and IL-2 and IFN-γ by CD8 or CD4 T cells generated after 5 hour in vitro stimulation of day 14 p.i. splenocytes with viral peptide. Representative plots are gated on IFN-γ^+ CD8 or CD4 T cells, and are from one of three mice.



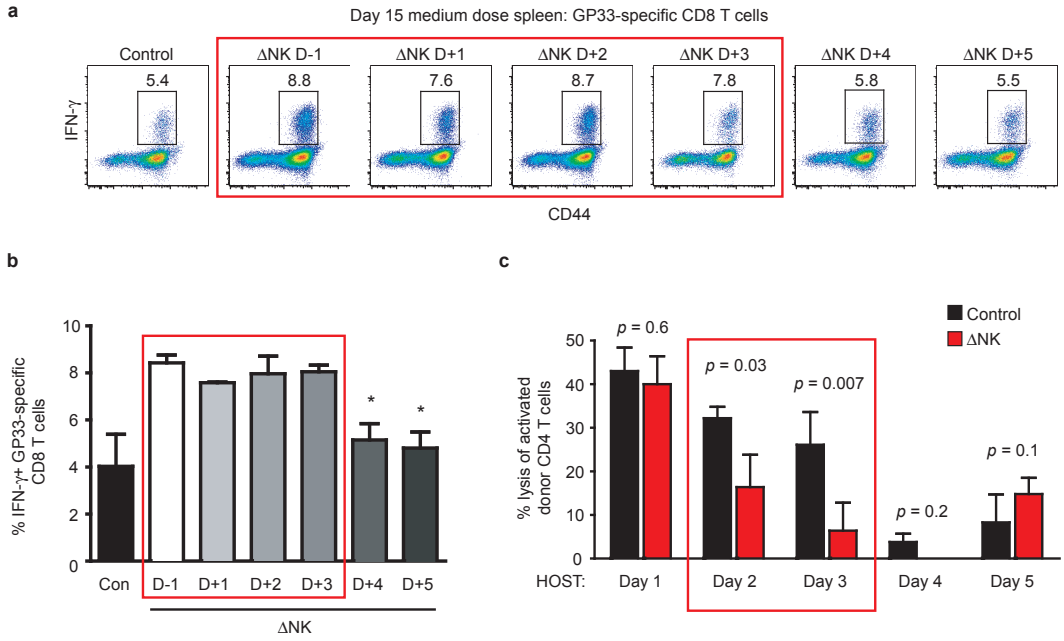
Supplemental Figure 5. Increased magnitude and enhanced in vivo CTL activity of LCMV-specific CD8 T cell responses in NK cell-depleted mice. a, Specific lysis of viral peptide-coated target cells in vivo during 16 hour assay at day 4 p.i. (2 x 10^6 PFU, high dose) in mice (n=8/group) treated with isotype (Control) or 25 μg anti-NK1.1 (ΔNK). **b**, Ly5.1$^+$ LCMV-specific P14 TCR transgenic CD8 T cells (1 x 10^4) were transferred i.v. into WT mice (n=3-4/group) one day before treatment with isotype (Control) or anti-NK1.1 (ΔNK). One day after NK cell depletion, mice were infected with a low dose (5 x 10^4 PFU) of LCMV clone 13 i.p. At day 6 p.i., donor P14 T cells (Ly5.1$^+$ CD8β$^+$ Vα2$^+$) were enumerated (mean±s.e.m) in various tissues.



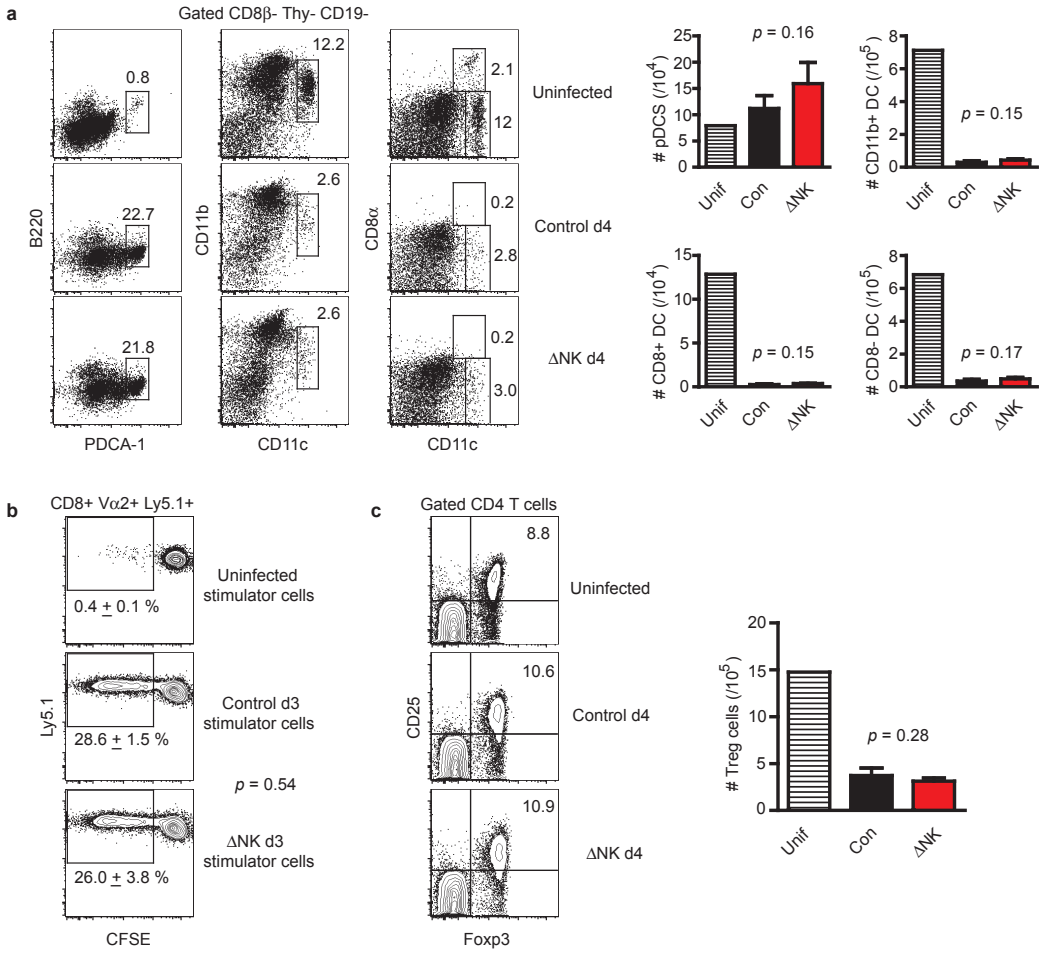
Supplemental Figure 6. Role of CD4 T cells in NK suppression of anti-viral CD8 T cell responses. Prior to medium (2 x 10^5 PFU) or high dose (2 x 10^6 PFU) infection, mice were injected with isotype (Control), anti-NK1.1 (ΔNK), anti-CD4 (ΔCD4), or both (ΔNKΔCD4). At day 12 p.i., cytokine co-production and total numbers of LCMV-specific IFN-γ^+ CD8 T cells were determined in the spleen (n = 3-5/group).



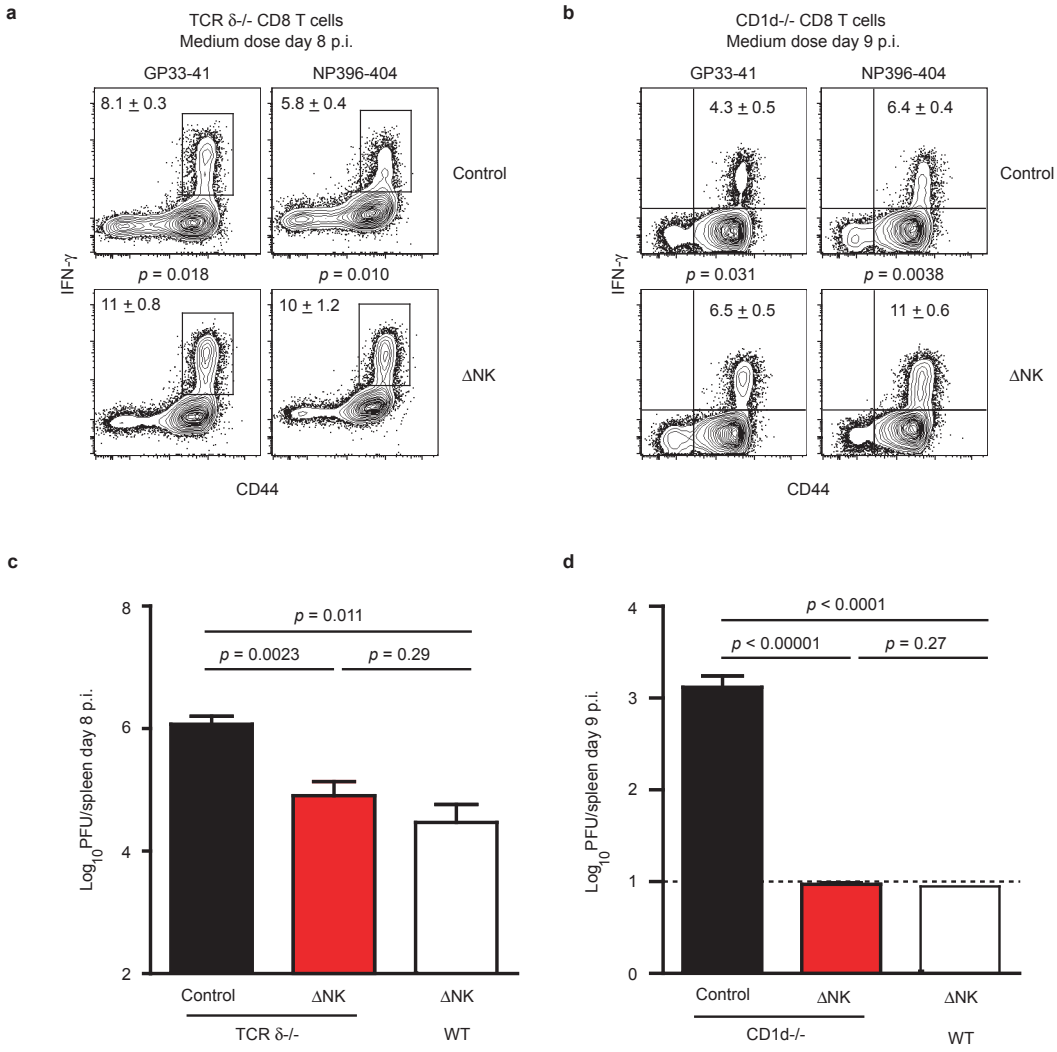
Supplemental Figure 7. Differential up-regulation of CD48 by activated CD4 and CD8 T cells. WT mice (Ly5.1^{+}, n=9) were treated with 25 μg anti-NK1.1 to deplete NK cells and infected one day later with medium dose (2 x 10^5 PFU) LCMV clone 13. At day 4 of infection, splenocytes were stained with antibodies specific for CD4, CD8α, CD44, CD43(1B11), and CD48. Mean fluorescence intensity of CD48 expression was determined on gated naïve phenotype (CD44^{low} CD43(1B11)^{neg}) or activated phenotype (CD44^{hi} CD43^{+}) CD4 and CD8 T cells. Representative overlay demonstrates differential expression of CD48 by naïve CD4 T cells (black line) and CD8 T cells (grey line) as well as by activated CD4 T cells (blue line) and CD8 T cells (red line).



Supplemental Figure 8. NK cells act within the first three days of LCMV clone 13 infection. **a-b**, Groups of C57BL/6 mice (n=3/group) were depleted of NK cells at day -1, +1, +2, +3, +4, or +5 relative to infection with a medium dose (2 x 10^5 PFU) of LCMV i.v. **a**, Representative plots and (**b**) mean proportion of IFN-γ+ GP33-41-specific CD8 T cells in spleen at day 15 p.i. Significant differences between "ΔNK D-1" and other groups of mice are denoted: **p*<0.05, ***p*<0.01. **c**, In vivo cytotoxicity assay demonstrating in vivo loss (mean±s.e.m, n=2-5/group) of activated ($CD44^{hi}CD43^{+}$) donor ($Ly5.1^{+}$) T cells from NK cell-depleted, medium dose-infected WT donor mice (day 4 p.i.) 5 h after transfer into control or NK-depleted (ΔNK) $Ly5.2^{+}$ mice infected one to five days previously with medium dose LCMV, relative to cells transferred into uninfected host mice. Red boxes denote "kinetic windows" of NK cell activity against T cells, defined as either (**a**) enhancement of subsequent CD8 T cell responses or (**b**) NK cell-dependent loss of activated CD4 T cells in the in vivo cytotoxicity assay.



Supplemental Figure 9. NK cell depletion does not alter antigen presentation or frequency of DCs and Tregs. **a**, Representative plots of lineage-negative ($CD19^-$ $CD8\beta^-$ Thy^-) splenocytes demonstrate frequency and total number of $CD8^+$ $CD11c^+$ DC, $CD8^-$ $CD11c^+$ DC, $CD11b^+$ $CD11c^+$ DC, and $PDCA\text{-}1^+$ $B220^+$ pDCs in spleen at day 4 of medium dose infection in control and ΔNK mice (n=3/group). **b**, $Ly5.2^+$ splenocytes (n=5/group) from uninfected mice as well as isotype-treated (Control) or anti-NK1.1-treated (ΔNK) medium dose (2×10^5 PFU) LCMV-infected (day 3 p.i.) mice were irradiated and used as stimulator cells (1:10 ratio) in an in vitro antigen presentation assay with CFSE-labeled $Ly5.1^+$ LCMV-specific TCR transgenic P14 CD8 T cells. After five days in vitro, P14 cells were analyzed for dilution of CFSE. **c**, Frequency (among $CD4^+$ T cells) and mean total number of $Foxp3^+$ $CD25^+$ Tregs in spleen of control and ΔNK mice (n=3/group) at day 4 p.i. Results presented as mean±SEM and *p* values denote significant differences between Control and ΔNK groups.



Supplemental Figure 10. γδ and NK T cells are dispensable for enhanced anti-viral T cell responses or viral clearance in absence of NK cells. Mice deficient in (**a** and **c**) γδ T cells (TCR γδ-/-) or (**b** and **d**) NK T cells (CD1d-/-) were treated with 25 μg IgG2a (control) or anti-NK1.1 (ΔNK) i.p. (n=5/group) one day prior to infection with a medium dose (2 x 10^5 PFU) of virus i.v. On day 8 (**a** and **c**) or day 9 (**b** and **d**) of infection, the (**a** and **b**) frequencies of IFN-γ-expressing LCMV-specific T cells and (**c** and **d**) viral load were determined in the spleen. Dotted line indicates limit of detection, and *p* values indicate significant differences between Control and ΔNK groups of mice.